REMARKS

Status of the Claims

Claims 1-5 are pending in this application, which is a continuation of U.S. Patent Application No. 09/763,117, wherein claims 1-4 and 9 were pending as of the date of abandonment. Applicant has amended claims 1-4, canceled claim 5 and added new claims 6-20. After claim amendments, cancellations and additions herein, claims 1-4 and 6-20 will be pending.

Comment Regarding Cited Documents

On September 9, 2004, along with the Office Action, the USPTO sent to Applicant a Notice of References Cited (Form PTO-892) and copies of the four (4) cited references. Applicant respectfully requests that the Examiner provide a copy of any Notice of References Cited (Form PTO-892) that was issued in the parent U.S. Patent Application No. 09/763,117, for example those provided by the USPTO with Office Actions on July 5, 2001 and April 19, 2002, so as to indicate that the references considered in that parent application were also considered in this application, in accordance with Manual of Patent Examining Procedure § 707.05(a).

With the September 9, 2004 Office Action, the USPTO also sent to Applicant a copy of an annotated Form PTO-1449 listing prior art cited that was filed by Applicant on January 7, 2002 with regard to parent U.S. Patent Application No. 09/763,117, and by a signature at the bottom the Examiner indicated that she considered the references listed thereon. Applicant notes that references AI, AJ and AN listed on that Form PTO-1449 are lined through with the notation "DUP", indicating that they were duplicates, i.e., that they had previously been considered by the Examiner on another Form PTO-1449 submitted by Applicant (in fact, they were listed on another Form PTO-1449 submitted by Applicant on March 20, 2002, and Applicant believes that this notation "DUP" was made by the previous Examiner Peter Paras in order to indicate this duplicate submission). Applicant respectfully requests an indication from the current Examiner that these lined-through references have been considered for this application, separate and apart from the parent application, in accordance with Manual of Patent Examining Procedure § 707.05(a).

Objection to Drawings

In the Office Action dated September 9, 2004, the Examiner objected to the drawings because Figures 3-7 and 9-14 are dark and the details of the images are difficult to see. In response, Applicant herewith submits eight (8) sheets of replacement formal drawings containing Figures 1-15 in place of the eight (8) sheets of drawings containing Figures 1-15 that were filed with the application.

Rejection Under § 101

In the September 9, 2004 Office Action, the Examiner rejected claims 1-2 under 35 U.S.C. § 101 as being directed to non-statutory subject matter, because the term "transgenic animal" encompasses a human being. In response to this rejection, Applicant has amended these claims to recite "non-human transgenic animal". As now amended, the claims overcome this rejection, and the rejection of the claims under 35 U.S.C. §101 should be withdrawn. Similarly, all new claims also recite the words "non-human" before the word "animal".

Rejections Under § 112

The Examiner rejected claims 1-5 under 35 U.S.C. § 112, first paragraph, on the grounds that the specification does not enable a person skilled in the art to make and use the invention as claimed. Specifically, with regard to claims 1-3, the Examiner contended that the specification, while being enabling for a transgenic mouse that expresses wheat germ agglutinin (WGA) under the control of the L7 or OMP promoter, does not reasonably provide enablement, without undue experimentation, for any other "transgenic animal" expressing a trans-synaptic protein, for any other trans-synaptic tracer protein, for any other promoter to drive expression in neurons or for any neural cells, other than ones that express trans-synaptic protein, under the control of the L7 or OMP promoter. The Examiner stated that, at the time of the instant application, the field of transgenic mammals, including the pronuclear injection method of generating transgenic animals, was unpredictable in that results obtained with one species cannot be predictive of results that would be obtained for another species.

With regard to claims 4-5, the Examiner stated that claim 4 is to a method of screening for neuromimetic substances and claim 5 is to a neuromimetic substance obtained from the

screen of claim 4, and nothing in the specification teaches one skilled in the art how to determine whether or not a test substance has an effect on a neuron via monitoring its expression of trans-synaptic protein, since the specification does not describe assays or how the neurons will be monitored to determine the effect of the neuromimetic substance.

Applicant first thanks the Examiner for her indication of allowable subject matter of a transgenic mouse whose genome comprises a nucleotide sequence encoding a trans-synaptic tracer protein, namely WGA, operably linked to a neuron specific promoter, wherein WGA is expressed in neurons of interest. Applicant has added new claims in order to more particularly define the invention so as to cover the subject matter stated by the Examiner as being allowable. Applicant believes that these claims are allowable.

Applicants traverse the Examiner's rejection of claims 1-3 as not being enabled. The first paragraph of 35 U.S.C. § 112 requires nothing more than objective enablement. Whether this is achieved by illustrative examples or by broad terminology is of no importance. *In re Marzocchi,* 169 USPQ 367 (CCPA 1971). An assertion by the USPTO that the enabling disclosure is not commensurate with the scope of the protection sought must be supported by evidence of reasoning substantiating the doubt so expressed. *In re Dinh-Nguyen,* 181 USPQ 46 (CCPA 1974); *In re Bowen*, 181 USPQ 48 (CCPA 1974); *In re Armbruster*, 185 USPQ 152 (CCPA 1975). In addition, it is improper to reject claims on the ground that the specification does not support the claims when the claims are no broader than the broadest description of the invention in the specification and there is no reason to challenge the operativeness of the subject matter embraced by the claims. *Ex parte Altermatt,* 183 USPQ 436 (POBA).

The enablement requirement is satisfied if the specification describes any method for making and using the claimed invention that bears a "reasonable correlation" to the entire scope of the claims. *In re Fisher,* 427 F.2d 833, 166 USPQ 18, 24 (CCPA 1970). In addition, although a patent must provide an enabling disclosure at the time the application was filed, the application need not contain within its four corners all of the information necessary to practice the claimed invention. Manual of Patent Examining Procedure § 2164.05 (a). Information that was well

known to those skilled in the art need not be included in the application and is preferably omitted. *In re Buchner,* 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed Cir. 1991).

According to the Examiner, the specification does not reasonably provide enablement for a "trans-synaptic tracer protein" in a "transgenic animal". The Examiner alleged that it would require undue experimentation to make and/or use the claimed invention *inter alia* because of the unpredictability of the art of transgenic animal generation. In response, Applicant herein amends claims 1-3, adds new claims 6-20, and submits Declarations of Tetsuo Noda and Hiroshi Suzuki Under 37 C.F.R. § 1.132 to overcome the Examiner's rejections. Amended claim 1 now recites "A transgenic non-human animal whose genome comprises a nucleotide sequence encoding a trans-synaptic tracer protein operably linked to a neuron specific promoter, wherein the trans-synaptic tracer protein is expressed in neurons of interest".

As specifically established by Dr. Tetsuo Noda in the Declaration of Tetsuo Noda submitted herewith, Applicant asserts that the expression of any trans-synaptic tracer protein not only in mice but in other animals could be achieved according to the description in the specification by a person skilled in the art without undue experimentation as of the date when the invention was made, as described below. Applicant notes that trans-synaptic tracer proteins, such as plant lectins described in the specification and nontoxic C-terminal fragment of tetanus neurotoxin (TTC), have conventionally been used for visualization of neural pathways in animals. For example, "trans-synaptic tracer" proteins are well known, as described in Yoshihara, *Neuroscience Research*, Vol. 44, pp. 133-40 (2002), and Horn et al., *Experimental Brain Research*, Vol. 81, pp. 353-62 (1990), copies of which are submitted herewith. See Declaration of Tetsuo Noda, ¶ 6.

Applicant also notes that these trans-synaptic tracer proteins have been injected into a variety of animals having a nervous system, including rodents such as mice, rats, guinea pigs; primates such as monkeys; and other mammals such as rabbits, cats; and birds. The following references describe injection of WGA into animals other than rodents: LaVail et al., Journal of Cell Biology, Vol. 96, pp. 373-81 (February 1983) (injection into bird); Tanaka et al., *Journal of Laryngology and Otology*, Vol. 107, pp. 916-19 (October 1993) (injection into cat); Pons et al.,

Journal of Comparative Neurology, Vol. 248, pp. 313-35 (1986) (injection into Cynomolgus monkey); and Jensen et al., *Brain Research*, Vol. 586, pp. 125-29 (1992) (injection into rabbit), copies of which are submitted herewith. See Declaration of Tetsuo Noda, ¶ 7.

The present invention enables specific expression of a trans-synaptic tracer protein in particular neurons, by operably linking a neuron-specific promoter and a gene encoding the trans-synaptic tracer protein. For example, as shown in the Examples, by ligating a gene encoding WGA downstream of L7 or OMP promoter, the specific expression of WGA in L7 promoter functioning mouse cerebellar Purkinje cell and OMP promoter functioning mouse olfactory nervous system, and the visualization of the neural pathway via transport of WGA from neuron to neuron, can be established. See Declaration of Tetsuo Noda, ¶ 8.

Horowitz et al., as reported in *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 96, pp. 3194-99 (March 1999), a copy of which is submitted herewith, generated a transgenic mouse in which a gene encoding barley lectin (BL) linked downstream of OMP promoter is introduced, and employed the mouse in experiments for visualizing neural pathways by detecting the BL. Horowitz et al. accomplished olfactory nervous system specific visualization of neural pathways employing BL, similar to that accomplished in the Examples of the present application using WGA. The Horowitz et al. reference demonstrates that the expression of trans-synaptic tracer proteins other than WGA in transgenic animals can provide the same effect. See Declaration of Tetsuo Noda, ¶ 9.

The Examiner also pointed out the difficulty in the selection of suitable promoters. However, neuron-specific promoters can function in a variety of host organisms in a similar manner. For example, OMP promoters, which are described in the specification and function specifically to olfactory neuron, were cloned from rats and mice, and are shown to function in olfactory neuron-specific manner. A transgenic mouse, in which a lacZ gene fused downstream of rat OMP promoter is introduced, expresses lacZ specifically to olfactory neurons, as reported in Forss-Pettera et al., *Neuron*, Vol. 5, pp. 187-97 (August 1990), a copy of which is submitted herewith. Applicants submit that this result clearly indicates that the rat neuron-specific promoter can function in a similar manner in mice. See Declaration of Tetsuo Noda, ¶ 10.

As described in the Examples in the specification, mouse-derived L7 promoter can express lacZ in rat, and the expression is specific to cerebellar Purkinje cells similarly as in mice. Applicants submit that, similar to OMP promoter, it is possible to express exogenous proteins in a wide range of host organisms employing this neuron-specific promoter. Further, L7 protein, which is naturally expressed by the L7 promoter, has been cloned from mice, rats, and human. The amino acid sequences thereof are highly conserved among species, and the proteins are functionally correlated and regulated so as to express specifically in cerebellar Purkinje cells, as reported in Zhang et al., *Molecular Brain Research*, Vol. 105, pp. 1-10 (2002), a copy of which is submitted herewith. See Declaration of Tetsuo Noda, ¶ 11.

In addition, the protein Olf-1 is known as a trans-acting regulator for OMP promoter, and the sequence of cis-acting element, to which the Olf-1 binds, has been found in the OMP promoter. It is possible to identify olfactory neuron specific promoters other than OMP and regulating proteins using common computer analysis based on these sequences, as reported in Wang et al., *Molecular and Cellular Biology*, Vol. 13, No. 9, pp. 5805-13 (September 1993), a copy of which is submitted herewith. See Declaration of Tetsuo Noda, ¶ 12.

Additionally, Maskos et al., as reported in *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 99, No. 15, pp. 10120-25 (July 2002), a copy of which is submitted herewith, generate a transgenic mouse in which a gene encoding a GFP-TTC fusion protein linked downstream of rat Calbindin promoter, which function in cerebellar Purkinje cells, is introduced. TTC is a protein known to be a trans-synaptic tracer, as described above by Horn et al. As shown by Maskos et al., the GFP-TTC fusion protein is expressed and visualized in the transgenic mouse, and the expression pattern is the same as in rats, showing not only that a trans-synaptic tracer protein other than WGA can be used in the present invention, but also that a rat neuron-specific promoter can function in mice in the same way. See Declaration of Tetsuo Noda, ¶ 13.

Tabuchi et al., as reported in *Journal of Neuroscience Research*, Vol. 59, pp. 94-99 (2000), a copy of which is submitted herewith, demonstrate that the present invention can be

applied to other animal species. Tabuchi et al. succeeded in visualizing optic pathways by expressing WGA operably linked via GAL4 to a Rhodopsin promoter (Rd1 promoter) in Dorosophila photoreceptor cells. Of course, various species of transgenic animals, such as rats, pigs, and chicks, were known in the art before the date when the invention was made as described in several publications. For example, transgenic rats could be obtained from suppliers such as Institute of YS New Technology Ltd., Tochigi, Japan or prepared according to Hirabayashi et al., "Transgene expression in mammary glands of newborn rats", Mol. Reprod. Dev. 43(2): 145-149 (1996), which was previously considered by the Examiner. See Declaration of Tetsuo Noda, ¶ 14.

The Examiner further contended that generation of transgenic animals using pronuclear microinjection is unreliable. However, as specifically established by Dr. Hiroshi Suzuki in the Declaration of Hiroshi Suzuki submitted herewith, Applicant states that transgenic mammals can be produced with high probability, and there are commercial suppliers for the production of transgenic rodents such as rats and mice (see, for example, Charles River Laboratories (www.criver.com) and Xenogen Biosciences Transgenic Technologies (www.xenogenbiosciences.com/index.html)). The development of pronuclear microinjection technology in mammals other than rodents was reviewed by Robert J. Wall in *Cloning and Stem Cells*, Vol. 3, No. 4, pp. 209-20 (2001), a copy of which is submitted herewith. See Declaration of Hiroshi Suzuki, ¶ 6.

The Examiner supported her allegation that the generation of transgenic non-murine animals using pronuclear microinjection is unreliable by referring to teachings by Mullins and Mullins (1996, J.Clin.Invest, 97: 1557-60). However, Applicant points out to the Examiner that the limitations cited by Mullins and Mullins regarding the application of pronuclear microinjection to non-murine animals are caused mainly by economics (see page 1557, second column, first full paragraph, lines 1-5), not by technical reasons that exist in the construction of non-murine transgenic animals alone. The other effects referred to by the Examiner, such as random integration in the host chromosome and position effect in the case of pronuclear microinjection, are commonly observed in the construction of transgenic animals, including mice. The difference between murine and non-murine animals in the construction of transgenic

animal is an efficiency of transgene integration and yield of transgenic offspring. See Declaration of Hiroshi Suzuki, ¶ 7.

In fact, the same authors, Mullins and Mullins, also published a review summarizing the result of transgenic animals other than mouse constructed at that time (see, Mullins and Mullins, *Hypertension*, Vol. 22, No. 4, pp. 630-33 (1993)), a copy of which is submitted herewith. In this review, it is reported that the frequency of integration onto chromosome in the construction of transgenic rabbit is slightly lower (12.8%) than that in mouse (27%) (see page 631, second column, the second full paragraph, line 11-18), referring to the pioneer study by Hammer et al. *Nature*, Vol. 315, pp. 680-83 (1985)), a copy of which is submitted herewith. See Declaration of Hiroshi Suzuki, ¶ 8.

Applicant states that the reproducible result for obtaining transgenic rabbits can be established with the common method of pronuclear microinjection even if its efficiency is slightly lower (12.8%) than the efficiency of transgenic mouse (27%) as seen in this review. Mullins and Mullins reported actual examples for generation of transgenic rats, rabbits, sheep, goats, pigs and cows in the same review (see the corresponding paragraph). Applicant points out that the establishment of transgenic animals other than mouse has been realized at appropriately reproducible frequency. Applicant believes that lower frequency (efficiency or yield) in the production of the transgenic animals does not constitute a reason for rejection, and that pronuclear injection technique is developed in mice with the highest level is mostly due to its the ease of operation and economy of using mice, as opposed to other animals, for this purpose. See Declaration of Hiroshi Suzuki, ¶ 9.

Accordingly, as discussed above, the Declaration of Tetsuo Noda submitted herewith shows that, based on the teachings of the specification, one skilled in the art could without undue experimentation produce the invention as claimed, i.e., that any transgenic animal with trans-synaptic tracer proteins other than WGA could be made without undue experimentation based upon the disclosures of the specification and the examples. Furthermore, the Declaration of Hiroshi Suzuki submitted herewith shows that, based on the teachings of the specification, one skilled in the art could without undue experimentation produce the claimed model animals, the

method of screening, and the production of transgenic animals other than mice. Applicant believes that the showings made in these Declarations Under 37 C.F.R. § 1.132 are sufficient to overcome the Examiner's enablement rejection under 35 U.S.C. § 112, first paragraph, and Applicant requests that this rejection be withdrawn.

In response to the rejection of claims 4-5 under 35 U.S.C. § 112, first paragraph, as not being enabled, claim 5 has been canceled and claim 4 have been amended to recite the steps of creating cultured neurons according to new claim 6, administering a test substance to a first group of the cultured neurons, determining the expression level of the trans-synaptic tracer protein in the first group of cultured neurons, determining the expression level of the trans-synaptic tracer protein in a second group of the cultured neurons as a control, and comparing the expression level of the trans-synaptic tracer protein in the first group to the expression level of the trans-synaptic protein in the second group, wherein a measurable difference in expression provides an indication of the effect of the test substance. These new steps are fully enabled in the specification, for example at page 5. Applicant believes that these amendments overcome the Examiner's rejections, and respectfully request that the rejections of these claims be withdrawn.

The Examiner also rejected claims 1-5 under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement, since the specification does not describe what is a "neuromimetic substance". In response, Applicant first notes that claims 1-3 contain no reference to the term "neuromimetic substance", and consequently this rejection of claims 1-3 is in error and should be withdrawn. Furthermore, Applicant has amended claim 4 so that the terms "neuromimetic substance" and "neuromimetic effect" are no longer recited. Now, claim 4 refers to the "effect" of the test substance that is indicated by the measurable difference in expression level. Claim 7 further defines the effect of the test substance as one that is chosen from among the group consisting of effect upon cell survival and maintenance, dendrite extension, synapse formation, enzymatic activity and neurotransmitter production, as set forth at page 5, lines 5-7 of the specification. Applicant requests that this rejection be withdrawn.

Claims 1-4 are also rejected under 35 U.S.C. §112, second paragraph, for being indefinite for failing to particularly point out and distinctly claim the subject matter that applicant regards as the invention. The Examiner states that claim 1 is confusing because it can be read as a gene in the form of mRNA or in the form of DNA. In response, Applicant has amended claim 1 to recite a transgenic non-human animal whose genome comprises a nucleotide sequence encoding a trans-synaptic tracer protein operably linked to a neuron specific promoter, wherein the trans-synaptic tracer protein is expressed in neurons of interest. Applicant believes that this amended terminology overcomes the Examiner's rejections and requests that this rejection be withdrawn. Claims 2-4 and new claims 6-20 depend from claim 1, and Applicant believes that these claims are also patentable.

Rejection Under § 102

The Examiner rejected claim 5, which is a product-by-process claim, under 35 U.S.C. § 102(b) as being anticipated by Curzon, et al. (1997, Trends in Pharmacological Science, 18: 21-25) or Elias, et al. (1998, Neuron, 21: 1375-1385). According to the Examiner, even though product-by process claims are limited by and defined by the process, determination of patentability is based on the product itself, not on its method of production. In this case, each of the references discloses a neuromimetic substance, thus anticipating claim 5. Applicant notes that claim 5 has herein been canceled, and this rejection is, therefore, moot.

Conclusion

In view of the amendments and arguments set forth herein, Applicant believes that all claims 1-4 and 6-20, as amended or presented herein, are in condition for allowance. In the event that the Examiner determines that the application is not in condition for allowance, Applicant respectfully requests that the Examiner contact the undersigned in order to resolve any outstanding issues by way of a telephone interview before another Office Action is issued in this application.

A favorable action on the merits is earnestly solicited.

Respectfully Submitted,
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Attachments